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REPORT DOCUMENTATION PAGE

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1. REPORT NUMBER: ARO 23953.7-LS	2. GOVT ACCESSION NO.: NA
3. RECIPIENT'S CATALOG NUMBER: NA	
4. TITLE (and Subtitle): Regulation of catabolic enzyme biosynthesis in Thermomonospora curvata	5. TYPE OF REPORT & PERIOD COVERED: Final report: 04-01-86 to 03-31-88
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Fred Stutzenberger, Department of Microbiology	8. CONTRACT OR GRANT NUMBER(s): DAAL03-86-K-0058
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Clemson University, Clemson, SC 29634-1909	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: NA
11. CONTROLLING OFFICE NAME AND ADDRESS: U. S. Army Research Office, P. O. Box 12211, Research Triangle Park, NC 27709-2211	12. REPORT DATE
	13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report): UNCLASSIFIED
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

NA

18. SUPPLEMENTARY NOTES

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19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

actinomycete; cellulase; cellulose; cyclic AMP; exoenzymes; phosphodiesterase; polygalacturonate lyase; cellobiose; beta-glucosidase; thermophile; Thermomonospora

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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20. ABSTRACT CONTINUED

The research studied under this contract included determination of the mechanisms by which the thermophilic actinomycete, Thermomonospora curvata, regulates the biosynthesis of catabolic exoenzymes at the levels of transcription, secretion, post-translational modification, and substrate-specific catalysis. The major results of this research are as follows: 1) We have demonstrated that T. curvata produces multiple forms of cyclic AMP phosphodiesterase (PDE), the enzyme responsible for the degradation of cyclic AMP. (we had previously shown that intracellular cyclic AMP level is one factor which controls the transcription rate of cellulase genes in the actinomycetes). Our paper is the first to describe PDE in any thermophilic actinomycete; furthermore, it is the first paper to demonstrate multiple forms of PDE in any bacterium and the first to demonstrate that PDE levels are influenced by growth phase, growth rate and carbon source. 2) We have described the strong preference that T. curvata shows for the uptake of cellobiose over that of glucose, the preferred carbon and energy source for most other bacteria. This disaccharide, which we have previously shown to be the sole degradation product of crystalline cellulose degradation by the actinomycete, is preferentially utilized even when T. curvata has been conditioned to grow on glucose alone and even when the glucose:cellobiose ratio is greater than 50:1. This preference for cellobiose utilization under all conditions tested is stronger than any shown for any other cellulolytic microbe and has important implications for the eventual application of the actinomycete as an enzyme source for large scale biomass conversion processes. 3) At the level of exoenzyme secretion, we have shown that the surfactant, Tween-80, effects a component-specific stimulation of cellulase release. Only components active against crystalline cellulose (as opposed to those active against soluble cellulose derivatives such as carboxymethyl cellulose) are stimulated; this stimulation was more apparent during growth on cellobiose octaacetate than on cellulose or its natural degradation product, cellobiose. 4) At the level of post-translational modification, we have shown that T. curvata cultures alter endoglucanase pattern during growth on cellulose. This finding correlates well with the earlier report by Calza et al. (Biochemistry 24:7797, 1985), who have suggested that an extracellular serine protease produced by Thermomonospora effects limited proteolysis of the extracellular endoglucanases, modifying both molecular and kinetic characteristics. 5) We have published the first report to describe a polygalacturonate lyase (PL) in any thermophilic actinomycete. Since pectin is an important constituent of plant biomass, the action of this pectinolytic enzyme augments the action of other exoenzymes which degrade natural plant fibers. The PL was purified to electrophoretic and serologic homogeneity and was characterized as to molecular weight, isoelectric point, amino acid composition, functional disulfide bonds, essential cofactors and optimal conditions for activity. Furthermore, we have employed a random-number-generating computer program to calculate the minimal susceptible site for PL attack on the pectin molecule. To our knowledge, this is the first application of such a program for determination of susceptible site size for any enzyme degrading any polymeric substrate. In summary, this research has been exceedingly productive given the meager level of funding. Five publications in international refereed journals have been produced, covering a wide range of factors which influence the biodegradative activities of Thermomonospora. Understanding the importance of such factors at the genetic and catalytic levels is essential to the eventual biotechnological application of these enzymatically versatile microbes.

PUBLICATIONS GENERATED BY THIS RESEARCH

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